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REGULAR ARTICLE

Neutrophil Count Is Associated With Reduced Gray Matter and Enlarged Ventricles in First-Episode Psychosis

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Although there is recent evidence that cells from the peripheral immune system can gain access to the central nervous system in certain conditions such as multiple sclerosis, their role has not been assessed in psychosis. Here, we aimed to explore whether blood cell count was associated with brain volume and/or clinical symptomatology. A total of 218 participants (137 first-episode psychosis patients [FEP] and 81 healthy controls [HC]) were included in the study. For each participant, a T_1 structural image was acquired, from which brain tissue volumes were calculated. We found that, in FEP, neutrophil count was associated with reduced gray matter (GM) volume ($\beta = -0.117$, $P < .001$) and increased cerebrospinal fluid volume ($\beta = 0.191$, $P = .007$). No associations were observed in HC. GM reduction was generalized but more prominent in certain regions, notably the thalamus, the anterior insula, and the left Heschl's gyrus, among many others. Neutrophil count was also associated with the total PANSS score ($\beta = 0.173$, $P = .038$), including those items assessing hallucinations ($\beta = 0.182$, $P = .028$) and avolition ($\beta = 0.197$, $P = .018$). Several confounders, such as antipsychotic medication, body mass index, and smoking, were controlled for. Overall, the present study may represent the first indirect evidence of brain tissue loss associated with neutrophils in psychosis, and lends support to the hypothesis of a dysregulated immune system. Higher neutrophil count was also associated with more severe clinical symptomatology, which renders it a promising indicator of schizophrenia severity and could even give rise to new therapies.

Key words: neuroimmunology/structural neuroimaging/leucocytes/hallucinations/avolition

Introduction

Despite it being one of the most devastating mental disorders, we are still far from knowing what factors are responsible for the beginning of schizophrenia and related disorders. One of the most promising theories proposes that the immune system is involved in the etiology of the disease, either due to immunological alterations or autoimmune mechanisms. In fact, there is a vast number of studies that have examined abnormalities in microglia,[1] the

first immunological defense of the central nervous system, in schizophrenia. However, data from postmortem studies have not yielded consistent results[1]; while some studies have found increased microglial cells in patients with schizophrenia,[2–4] others have not found differences between patients and healthy participants.[5,6] Similarly, while some studies have found differences in microglial activation between patients with schizophrenia and healthy controls (HCs), the most recent research contradicts previous findings.[1] Furthermore, some research has focused on cytokines, which are small proteins that regulate inflammatory responses.[7] In this regard, several cytokines have consistently been shown to be increased in schizophrenia.[8,9]

Although available evidence is still controversial, it is plausible to think of an association between immunological factors and schizophrenia; however, not much research on the mechanisms by which the immunological alterations may affect the schizophrenic brain has been conducted. Kenk et al[10] did not find an association between microglial activation and the volume of some brain regions such as the hippocampus and the prefrontal cortex. The rest of the evidence available in this respect comes from studies that have analyzed either microglial activation or brain structural changes, but not both in the same sample; while in certain regions microglia seem to be related to structural changes, results are inconsistent and no conclusive evidence has been assembled.[1] Additionally, some recent studies have reported a relationship between cytokines and brain structure. Cannon et al[11] reported an association between inflammatory cytokines and prefrontal cortical thinning in patients at clinical high risk who converted to psychosis. In schizophrenia, higher levels of inflammatory cytokines have been found associated with reduced volume of some brain areas that were previously selected as regions of interest, such as Broca's area[12] and total cortical gray matter (GM) and superior frontal gyrus in a postmortem study.[13]

The role of the white blood cells (leucocytes), which form the peripheral immune system and are crucial in inflammatory processes, has been laid aside in the context of brain structural changes in schizophrenia. The fact that the blood–brain barrier (BBB) restricts the entrance of peripheral immune cells to the brain may explain this lack of interest. However, there is now increasing evidence that, under certain pathological conditions, the BBB may be disrupted and allow peripheral blood cells to enter the brain, as has been observed in multiple sclerosis (MS) and after stroke.[14] Importantly, it has been suggested that BBB may also be disrupted in schizophrenia.[15] So far, several studies have addressed the effects of leucocytes on an injured central nervous system. For an extensive review of this topic, see Gadani et al.[16] For example, neutrophils appear to exert destructive actions in the cerebral tissue after they infiltrate the brain, as has been reported in an animal model of traumatic brain injury.[17] Similarly, in humans that have suffered a stroke, neutrophil infiltration into the brain seems to be associated with larger infarct volumes and worse outcomes.[18] More controversy exists regarding monocytes and lymphocytes;[16] monocytes, for example, have been reported to cause neuronal damage in mice with induced seizures,[19] but also to protect the brain after a stroke.[20] In the case of lymphocytes, most of the research points predominantly to their protective effects in the brain,[16] as shown for instance by Marsh et al,[21] who reported an accelerated progression in a mouse model of Alzheimer's disease (AD) that lacked lymphocytes. However, whether lymphocytes have beneficial or detrimental effects after a stroke is still not clear.[22] Given that first-episode psychosis (FEP) patients present a progressive loss of GM volume,[23] it could be hypothesized that brain tissue loss may be due to the deleterious effects of peripheral white blood cells on the central nervous system. Therefore, we aim to investigate whether or not white blood cells are implicated in schizophrenia, and, if so in what manner. In addition to leucocytes, erythrocytes[24] and platelets[25] seem to play a part on immunity and will also be explored.

Here, we report a thorough analysis of whether and how blood cells are associated with brain volume in FEP patients and in HC participants. We employed a relatively large sample and a whole-brain approach to detect potential effects in an unbiased manner. First, we sought for differences between FEP and HC in regard to brain volume and blood cell count. Second, we examined whether any type of blood cell was associated with GM, white matter (WM), and cerebrospinal fluid (CSF) volumes in FEP and HC, and which specific brain regions were involved. Finally, we explored potential associations between blood cells and clinical symptomatology.

Methods

Participants

A total of 218 participants, 137 FEP patients and 81 HC participants, were selected from the larger sample of the PEPs study[26] if they were aged 13 or older, had a T_1 structural image of the brain, a blood sample, and all the demographic and clinical data available. Detailed information on how participants were recruited, inclusion/exclusion criteria, and ethical statements are available in the supplementary material.

Neuroimaging Data Acquisition, Processing, and Analysis

A high-resolution T_1-weighted structural image was obtained for each participant, who were scanned in 1 of the 5 scanners available for the PEPs study. Detailed information on data acquisition is available in the supplementary material. All neuroimaging data were processed and analyzed with SPM12 (Wellcome Department of Imaging Neuroscience, London; www.fil.ion.ucl.ac.uk/spm) running under MATLAB (Release 2012b, The MathWorks, Inc.). GM, WM, and CSF were segmented

for each participant employing the CAT12 toolbox (http://www.neuro.uni-jena.de/cat). We ensured that the images employed for the segmentation were of good quality by means of the internal tool provided by CAT12 to this end. The modulated and warped GM segments ("mwp1" files) derived from the segmentation were smoothed using a Gaussian kernel of 12 mm full-width-at-half-maximum and employed in the whole-brain voxel-based morphometry (VBM) analyses described in the "Statistical analysis" section. Additionally, the volume of specific brain structures was automatically calculated after the segmentation using the Neuromorphometrics atlas (http://www.neuromorphometrics.com). Total brain volume (TBV) was obtained from the sum of GM, WM, and CSF volumes.

Blood Sampling

Blood extraction was carried out in the baseline visit, and 2 months, 6 months, 1 year, and 2 years after the baseline visit. All blood samples were collected between 8:00 and 10:00 AM after an overnight fast. Since not all blood extractions and neuroimaging scans were carried out on the same day, blood cell count was derived either from the baseline or 2-month-visit blood sample according to the criteria presented in the supplementary material. The median days of difference between blood sampling and MRI were 14.5 for FEP and 17 for HC.

Demographic and Clinical Data

Information regarding age, sex, years of education, and body mass index (BMI) was collected for all the participants. The Positive and Negative Syndrome Scale (PANSS)[27] and the Symptom Onset in Schizophrenia scale (SOS)[28] were administered to FEP patients.

Medication and Substance Use

We collected information about the cumulative amount of antipsychotic medication taken daily by FEP patients, from the first day of medication until the day on which the blood extraction was carried out, as well as whether they were taking other psychotropic medications, namely anxiolytics, antidepressants, lithium, other mood stabilizers, and biperiden. Nonantipsychotic medication intake was required to start at least 1 week before the blood extraction to be considered. Moreover, the number of cigarettes of tobacco and/or cannabis smoked daily by all the participants was also recorded. Detailed information on how the amount of antipsychotic medication was calculated is available in the supplementary material.

Backward Elimination With Bootstrap Resampling Procedure

Aiming to determine which blood cell type was most significantly associated with our variables of interest (brain volume and clinical symptomatology), we employed a statistical procedure which combines the backward elimination method with bootstrap resampling, as proposed by Austin and Tu.[29] For each variable of interest, some predictors were tested, including the 7 blood cell type counts and several sociodemographic and clinical covariates. The backward elimination method was used in each bootstrap sample to generate a predictive model. This procedure was carried out automatically with the boot.stepAIC function (https://www.rdocumentation.org/packages/bootStepAIC/versions/1.2-0/topics/boot.stepAIC) of the R software (https://www.r-project.org). Later on, we determined the frequency with which each predictor was identified as significantly associated with the variable of interest. Following Austin and Tu,[29] some candidate models were then generated according to the frequency with which each variable was selected. Linear regression analyses were then conducted to test these candidate models. The first model to be tested was the one including the most frequently selected blood cell type, along with those covariates selected in all the bootstrap samples. Next, covariates selected in at least 90% of the bootstrap samples were added to the model to see if the previous one was significantly improved. This was repeated with the covariates selected in at least 80%, 70%, 60%, and 50% of the bootstrap samples. If a model significantly improved the previous one but one or more of the variables included in it were nonsignificant, these variables were removed and the remaining model was retested again. Eventually, this allowed us to identify the most parsimonious model. Finally, the rest of the blood cells were added, one at a time, to the final model to check whether the model fit was significantly improved.

Statistical Analysis

First, comparisons between FEP and HC regarding demographic and clinical data were conducted. Brain tissue volumes and blood cell count were also compared between FEP and HC with ANCOVAs controlling for age, sex, TBV, and years of education where appropriate. Second, the backward elimination with bootstrap resampling procedure explained above was conducted with GM, WM, and CSF volumes as dependent variables, and the 7 blood cell type counts (neutrophils, eosinophils, basophils, lymphocytes, monocytes, erythrocytes, and platelets), along with age, sex, years of education, TBV, antipsychotic medication, tobacco smoking, cannabis smoking, and BMI, as predictors. A whole-brain multiple regression VBM analysis was then conducted on GM tissue, including as predictors those variables that were identified as significantly associated with GM volume in the model-generating procedure. An absolute threshold masking corresponding to 20% tissue probability was employed, aiming to rule out voxels with a low probability of actually representing GM. An uncorrected

voxel-level cluster defining threshold P <.001 and a family wise error-corrected cluster-level threshold P <.05 were employed. A less-restrictive VBM analysis, employing an uncorrected voxel-level cluster defining threshold P <.001, without further correction for multiple comparisons, was conducted to identify brain regions likely to show global, rather than localized, effects. The whole volume of these brain regions, along with the whole volume of lateral, third, and fourth ventricles, were calculated and included in linear regression analyses, including as predictors those variables previously identified as significantly associated with GM volume. Third, backward elimination with bootstrap resampling analyses was again carried out, this time with the total PANSS score and the 3 PANSS subscale scores as dependent variables, and the 7 blood cell type counts, age, sex, years of education, TBV, antipsychotic medication, tobacco smoking, cannabis smoking, and BMI, as predictors. Linear regression analyses of the individual items were then conducted only with those variables that emerged as significant in the model-generating procedure. Lastly, some additional analyses to rule out effects from potential confounders, such as nonantipsychotic medication or the use of multiple scanners, were conducted. Since all these analyses were mostly exploratory and hypothesis-generating, no correction for multiple comparisons was performed unless otherwise specified.[30]

Results

FEP and HC Comparison

Demographic and clinical data are described and compared between FEP and HC in table 1. There were significant differences in years of education, and tobacco and cannabis smoking, but not in age, sex distribution, and BMI. Moreover, FEP had a smaller whole GM volume than HC, but larger global CSF, which included larger lateral (P = .023) and third (P = .010) ventricles. No differences between groups were observed for WM (table 1). Concerning blood cells, neutrophil count was significantly higher in FEP, whereas erythrocyte count was significantly higher in HC. No other differences in blood cell count were found (table 2).

Blood Cells and Brain Volume

In FEP, neutrophil count was found to be significantly negatively associated with GM volume in 98.4% of the bootstrap samples, more than any other blood cell type. Subsequent regression analyses confirmed the significant association between neutrophil count and GM volume, and allowed us to identify the most parsimonious model, which included neutrophil count (β = −0.117, P < .001), age (β = −0.251, P < .001), and TBV (β = 0.818, P < .001). Neutrophils were also the most selected (89.2%) blood cell type in association with CSF volume, although in this case the association was positive. Once again, the final model included neutrophil count (β = 0.191, P = .007), age (β = 0.166, P = .018), and TBV (β = 0.641, P < .001). On the other hand, none of the blood cell types was significantly associated with WM, which showed associations only with age (β = 0.207, P < .001) and TBV (β = 0.892, P < .001). As for HC, only age and TBV were associated with GM and WM volume, while years of education and TBV were associated with CSF volume. None of the blood cell counts was significantly associated with GM, WM, or CSF in HC. Detailed information on all these analyses is available in the supplementary material. The whole-brain VBM analysis in FEP showed neutrophil count to be consistently and specifically associated with reduced volumes of the right anterior insula, right temporal pole, right entorhinal area, right middle temporal gyrus, and right inferior temporal gyrus (figure 1 and supplementary table 1), after controlling for age and TBV. Afterwards, less specific linear regression analyses were conducted between neutrophil count and the whole volume of some brain regions, which included the aforementioned ones and additional brain regions that were identified in a less-restrictive VBM analysis (supplementary table 1). These analyses, which were carried out to search for more global effects, yet restricted to certain brain areas, showed neutrophil count to be associated with reduced whole bilateral volume of the thalamus (β = −0.211, P = .005), entorhinal area (β = −0.194, P = .002), anterior insula (β = −0.133, P = .019), among others, after controlling for age and TBV. Conversely, neutrophil count was associated with larger lateral (β = 0.196, P = .010) and third (β = 0.152, P = .062) ventricles (figure 2 and table 3).

Blood Cells and Clinical Symptomatology

Neutrophil count was significantly directly associated with total PANSS score in 78.4% of the bootstrap samples, more than any other blood cell type. The subsequent regression analyses yielded a model that confirmed the significant association between neutrophil count (β = 0.173, P = .038) and total PANSS score. Cannabis smoking (β = 0.245, P = .004) and years of education (β = −0.187, P = .030) were also significantly associated with the total PANSS score. Neutrophil count was also the most selected blood cell type in association with the positive (76.1%) and the general (75.7%) PANSS subscales. Additional regression analyses gave rise to the most parsimonious models, which included neutrophil count (β = 0.166, P = .046), years of education (β = −0.218, P = .009), antipsychotic medication (β = −0.250, P = .003), and BMI (β = −0.200, P = .015) in association with the positive subscale, and neutrophil count (β = 0.152, P = .064) and cannabis smoking (β = 0.296, P < .001) in association with the general subscale. Although the association between neutrophil count and the general subscale was seen to be at a trend-level, it was decided to include neutrophil count in the subsequent

Table 1. Demographic, Clinical Data, and Brain Tissue Volumes Are Compared Between First-Episode Psychosis (FEP) Patients and Healthy Control (HC) Participants

Variable	FEP (n = 137)	HC (n = 81)	P value
Age (years)	23.03 (6.06) [13–35]	23.75 (5.59) [14–35]	.381[a]
Sex	90 men/47 women	52 men/29 women	.823[b]
Years of education	12.27 (3.36) [7–24]	14.52 (3.28) [6–23]	**<.001**[a]
Ethnicity			.733[b]
Caucasian (no.)	122	70	
Hispanic (no.)	7	6	
Others (no.)	8	5	
Antipsychotic medication (mg/day)[c]	41.62 (68.53) [0–319.93]		
Risperidone (yes/no)	51/86		
Olanzapine (yes/no)	43/94		
Paliperidone (yes/no)	19/118		
Aripiprazole (yes/no)	18/119		
Quetiapine (yes/no)	11/126		
Clozapine (yes/no)	7/130		
Amisulpride (yes/no)	6/131		
Ziprasidone (yes/no)	3/134		
Haloperidol (yes/no)	1/136		
Perphenazine (yes/no)	1/136		
Zuclopenthixol (yes/no)	1/136		
Anxiolytics intake (yes/no)	40/97		
Antidepressants intake (yes/no)	17/120		
Lithium intake (yes/no)	10/127		
Other mood stabilizers intake (yes/no)	5/132		
Biperiden intake (yes/no)	18/119		
Days from psychosis onset to scan[d]	184.37 (121.62) [0–537]		
Total PANSS score	67.54 (25.93) [30–158]		
Positive PANSS score	16.47 (8.36) [7–41]		
Negative PANSS score	16.74 (8.39) [7–43]		
General PANSS score	34.34 (13.48) [16–82]		
Body mass index	23.69 (4.02) [16.44–36.80]	23.37 (2.97) [17.63–30.74]	.451[e]
Tobacco smoking (cigarettes/day)	7.72 (8.41) [0–40]	1.54 (3.84) [0–20]	**<.001**[e]
Cannabis smoking (cigarettes/day)	1.45 (3.49) [0–20]	0.05 (0.22) [0–1]	**<.001**[e]
Gray matter volume (mm^3)	712.18 (81.54) [521.28–960.11]	726.99 (70.84) [575.61–929.01]	**.007**[f]
White matter volume (mm^3)	523.68 (65.81) [374.97–713.22]	530.47 (56.91) [382.35–691.34]	.833[f]
Cerebrospinal fluid volume (mm^3)	275.79 (42.69) [186.07–388.68]	269.57 (48.53) [178.98–373.65]	**.027**[f]

Note: Data are expressed as mean (SD) [range]. Statistically significant differences are marked in bold.
[a]*P* value derived from a *t*-test.
[b]*P* value derived from a χ^2 test.
[c]Chlorpromazine equivalents estimation.
[d]Time between the date of onset of the first positive psychotic symptom, namely hallucinations or delusions (assessed with the Symptom Onset in Schizophrenia [SOS] scale) and the date of the scan.
[e]*P* value derived from an ANCOVA test controlling for age and sex.
[f]*P* value derived from an ANCOVA test controlling for age, sex, total brain volume, and years of education.

analyses to further explore its potential association with the individual items of this subscale. On the other hand, no significant associations arose between any blood cell type and the negative PANSS subscale, which was only associated with years of education (β = −0.264, P = .002). Detailed information on these analyses is available in the supplementary material. Additional analyses of the individual items of the positive and general subscales were carried out only with neutrophil count along with those variables that arose as significant in the previous analyses. These analyses revealed that neutrophil count was associated with the hallucinatory behavior (β = 0.182, P = .028), suspiciousness/persecution (β = 0.216, P = .010), hostility (β = 0.247, P = 0.005), disturbance of volition (β = 0.197, P = .018) and preoccupation (β = 0.283, P < .001) items (table 4).

Other Potential Confounders

To rule out potential confounding effects of the medications other than antipsychotics taken by FEP, all the dichotomous variables (yes/no) for each type of medication were included separately in all the analyses previously described. None of the previously reported associations between neutrophil count and brain volume and clinical symptomatology was significantly altered by the

Table 2. Leucocyte ($\times 10^9$/L), Erythrocyte ($\times 10^{12}$/L), and Platelet ($\times 10^9$/L) Count Comparison Between First-Episode Psychosis Patients (FEP) and Healthy Control (HC) Participants, by Means of an ANCOVA Controlling for Age and Sex

Blood Cell	FEP (n = 137)	HC (n = 81)	P Value
Neutrophils	4.08 (1.53)	3.66 (1.29)	**.025**
Eosinophils	0.35 (0.77)	0.32 (0.50)	.813
Basophils	0.06 (0.15)	0.09 (0.22)	.308
Monocytes	0.71 (1.17)	0.52 (0.51)	.198
Lymphocytes	2.22 (0.71)	2.20 (0.62)	.892
Erythrocytes	4.74 (0.48)	4.87 (0.43)	**.007**
Platelets	235.75 (51.48)	247.15 (52.70)	.153

Note: Data are expressed as mean (SD). Uncorrected P values are presented. Statistically significant differences are marked in bold.

inclusion of these variables. Even though lithium intake was significantly associated with increased GM volume ($\beta = 0.094$, $P = .003$), the association between neutrophil count and GM volume remained intact ($\beta = -0.121$, $P < .001$). Furthermore, and to ensure that blood cell levels were not artificially increased in patients due to an acute infection, bivariate correlation analyses were performed analyzing neutrophil count between different temporal moments in which blood was sampled. Neutrophil count was significantly correlated between the baseline, 1-year, and 2-year extractions (baseline-1y [$\beta = 0.375$, $P < .001$]; baseline-2y [$\beta = 0.417$, $P < .001$]). Likewise, neutrophil count was still significantly increased in FEP with respect to HC after 2 years (FEP [$n = 96$]: $4.21 \pm 1.46 \times 10^9$/L; HC [$n = 61$]: $3.41 \pm 1.07 \times 10^9$/L, $P < .001$). Finally, since we observed that the participants scanned in one of the centers were significantly younger and less educated than the rest (supplementary material), we repeated the brain volume analyses excluding the participants from this center to rule out potential confounding effects. The associations between neutrophil count and GM ($\beta = -0.100$, $P = .004$) and CSF ($\beta = 0.240$, $P = .001$) volumes were still significant.

Discussion

This is the first study to show an association between reduced GM volume and neutrophil count in the context of a psychotic disorder. To the best of our knowledge, this is also among the first studies in which reduced brain tissue is associated with neutrophils in humans. Interestingly, reduction in GM was found to be accompanied by increased CSF volume. These GM and CSF changes were only observed in FEP and not in HC, and were associated only with the amount of neutrophils, and, expectedly, age and TBV. No other blood cell types showed an association. Interestingly, neutrophils were the only blood cell type to be significantly increased in FEP when compared with HC. This is consistent with and extends a recent publication in which increased neutrophil count in nonsmoking, drug-naïve, FEP patients was reported.[31] Conversely, erythrocyte count was found to be increased in HC with respect to FEP.

A thorough analysis of the GM regions showing reduced volume associated with neutrophils on patients indicated that reduced volume was generalized rather than localized, ie, it was observed all over the brain. While specific, consistent reduced GM was observed in the anterior insula, the temporal pole, the entorhinal area, and the middle and inferior temporal gyri of the right hemisphere, less specific volume decrease was found in several other brain regions or structures when considering their whole bilateral volume, namely, the thalamus, the entorhinal area, the precentral gyrus, and the left Heschl's gyrus, among many others. Interestingly, neutrophil count was a better indicator of reduced volume than age in several regions. On the other hand, CSF was found to be increased in association with neutrophil count in FEP; the enlargement of the lateral and third ventricles seemed to account for most of this CSF increment. Altogether, this is suggestive of a volume tradeoff between GM and CSF. Actually, that enlarged ventricles are associated with GM loss in schizophrenia has been already reported,[32,33] but Gaser et al[33] observed that ventricle enlargement was associated not only with diffuse brain atrophy, but also with the atrophy of adjacent structures, particularly the thalamus, which is consistent with our observation of reduced volumes of the thalamus and other GM regions not adjacent to the ventricles. Even though the global GM volume reduction is the most relevant aspect of these results, it is worth mentioning that some of the regions whose reduced volume was associated with neutrophil count might be of special importance in psychosis. For example, the left Heschl's gyrus shows a progressive deterioration over time in first-episode schizophrenia patients,[34] and it appears to activate during auditory hallucinations in patients with schizophrenia.[35]

Although the observational nature of this study does not allow us to conclude that neutrophils are causing brain tissue loss, neutrophils have been associated with larger infarct volumes after stroke,[18,36,37] which is among the few indirect proofs available of brain tissue loss associated with neutrophils in humans. There is more evidence available from studies with animals, such as that of Kenne et al,[17] who showed that early neutrophil depletion reduced tissue loss secondary to the injury in a mouse model of traumatic brain injury. The importance of neutrophils in the progression of experimental autoimmune encephalomyelitis, an animal model of MS, has also been demonstrated.[38] Other studies employing rat models of traumatic brain injury and experimental autoimmune encephalomyelitis have shown that neutrophils can gain access to the central nervous system by infiltrating the choroid plexus of the ventricles,[39–41] which is

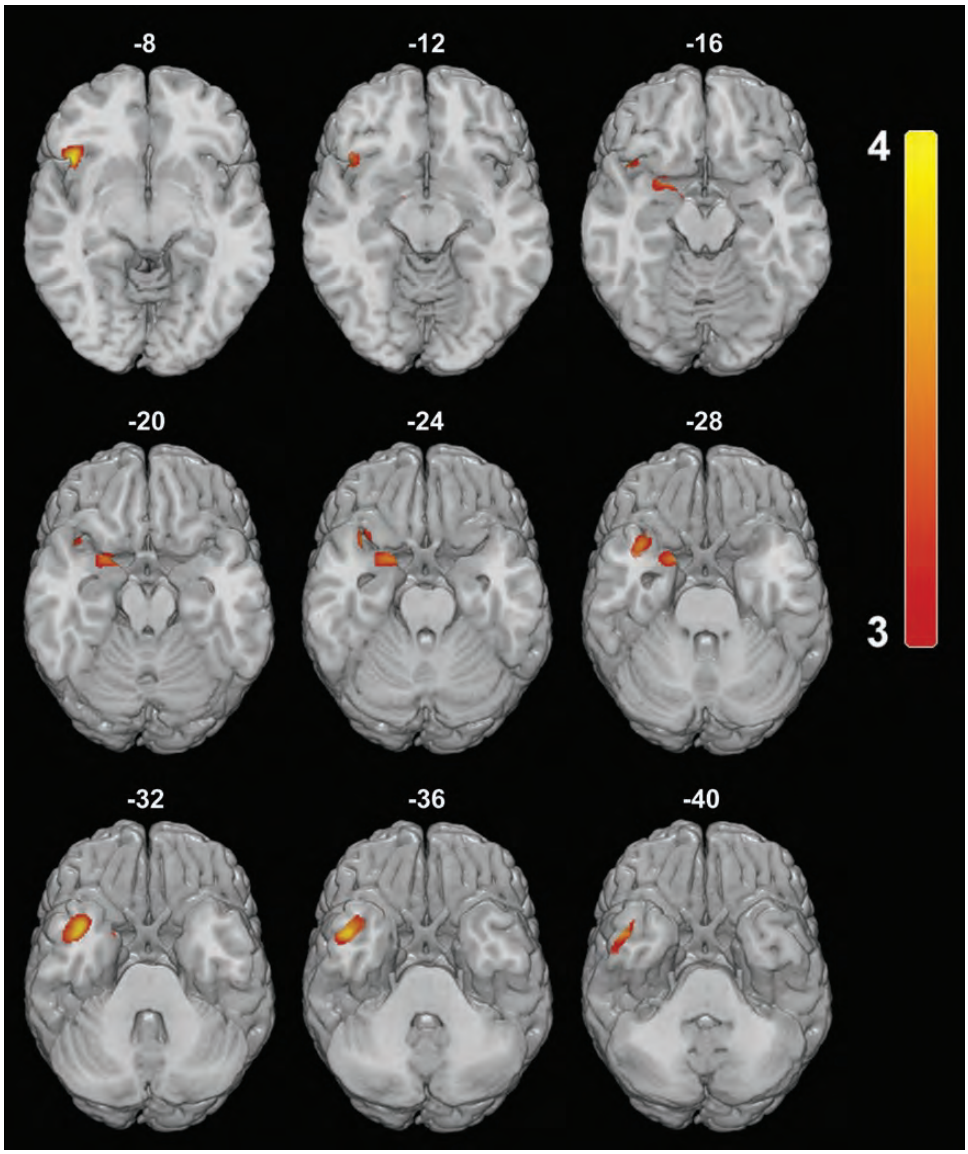

Fig. 1. Multiple basal slices are depicted showing the specific regions in which reduced volume was found to be associated with increased neutrophil count in first-episode psychosis, as identified in a voxel-based morphometry analysis employing an uncorrected voxel-level cluster defining threshold $P < .001$ and a family wise error-corrected cluster-level threshold $P < .05$. Top numbers indicate the "z" MNI coordinate. See supplementary table 1 for a list of these regions.

consistent with the ventricle enlargement observed here. Similarly, some studies have reported accumulation of neutrophils in the central nervous system in mouse models of the Alzheimer's disease.[42,43] In this regard, neutrophil depletion was shown to improve memory once the disease had started, and neutrophil blockade during the early stages of the disease was associated with improved cognitive function later on.[43] The present study represents, therefore, the first indirect proof of brain tissue loss associated with neutrophils in psychosis.

Higher neutrophil count was also associated with higher PANSS scores and, in particular, with some of the PANSS items from the positive and general subscales, such as hallucinatory behavior, suspiciousness, hostility, disturbance of volition, and preoccupation, among others. It is important to note that avolition is considered as a core negative symptom in schizophrenia,[44] although in the PANSS it is included in the general subscale instead of the negative subscale, which is probably inaccurate according to an analysis of the PANSS factor structure.[45] Therefore, neutrophil count appears to be a good indicator of the severity of some of the most important symptoms presented by FEP and schizophrenia patients, such as hallucinations and avolition. Interestingly, more years of education and higher BMI were associated with less severity of some of the symptoms, which may be studied in depth by future studies, although similar results with BMI have been already published.[46,47] Conversely,

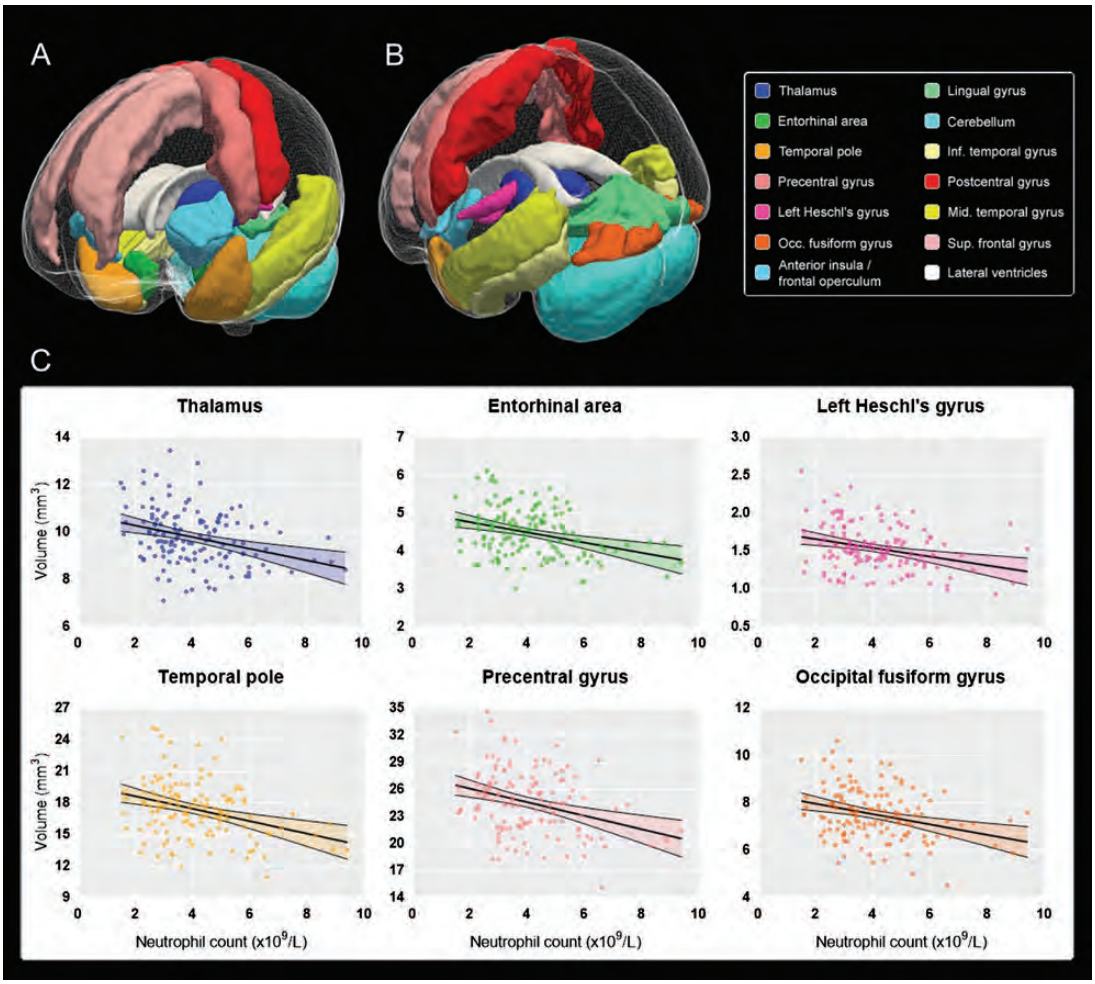

Fig. 2. (A) Anterolateral and (B) posterolateral views of a 3-dimensional schematic representation of the brain regions whose whole volume was found reduced in first-episode psychosis (FEP) patients associated with increased neutrophil count, except the lateral ventricles, which were found enlarged in association with higher neutrophil count. In C, scatter plots for FEP patients depicting the volume reduction of the most relevant regions are presented. See table 3 and supplementary table 1 for more information.

cannabis smoking was associated with increased severity of several symptoms.

The role of neutrophils in chronic, and not only acute, inflammatory diseases, as well as in neurological disorders such as MS or AD, has been an emerging research topic in recent years.[14,48,49] Our results support the idea of a dysregulated immunological system in schizophrenia, in which neutrophils would be the principal actors of the immunological scene. The finding of a decreased erythrocyte count in FEP compared with HC, even though the mean erythrocyte count of patients is within the normal parameters, lends further support to the idea of immunological alterations.[50] Moreover, if we assume that neutrophils act against GM tissue, that would also support the autoimmune hypothesis in schizophrenia. Even though neutrophil count is increased in FEP as compared with HC, it is unlikely that this factor alone would explain why patient brains are affected by neutrophils whereas control brains are not. Rather, structural or functional impairments of the brain and/or the immune system could provide a better explanation for this apparent neutrophil attack against its own host. For example, disruption of the BBB has been observed in MS and AD, and has been deemed as the probable cause of leucocyte entry into the brain.[51,52] Indeed, BBB disruption could be caused by neutrophils themselves.[43] The BBB also appears to be disrupted in schizophrenia.[15] Taken together, our results may reflect neutrophil infiltration in the brain through an impaired BBB, although this needs to be specifically corroborated in future studies. It is worth noting that some studies have shown an increase in BBB permeability in rats after acute stress exposure,[53,54] although other reports contradict previous findings (see, eg, the study by Roszkowski and Bohacek).[55] Therefore, stress may be an important factor to be considered as potentially responsible for BBB alterations. In addition, increased numbers of neutrophils have been seen both in humans and in mice exposed to chronic stress.[56]

Table 3. Linear Regression Analyses Between Neutrophil Count and the Whole Volume of Some Relevant Brain Regions of First-Episode Psychosis Patients That Were Identified in Previous Voxel-Based Morphometry Analyses

Brain Area	Neutrophil Count	Age	Total Brain Volume	R^2	*F* Test	*P* Value
Gray matter						
Entorhinal area	**−0.194***** (FDR)	−0.044	**0.641******	.512	46.585	<.001
Inferior temporal gyrus	**−0.127***** (FDR)	**−0.149******	**0.812******	.792	168.527	<.001
Temporal pole	**−0.168***** (FDR)	**−0.134****	**0.688******	.609	68.966	<.001
Precentral gyrus	**−0.157***** (FDR)	**−0.171*****	**0.705******	.652	82.891	<.001
Thalamus	**−0.211***** (FDR)	−0.111	**0.449******	.324	21.244	<.001
Middle temporal gyrus	**−0.117***** (FDR)	**−0.206******	**0.789******	.787	163.769	<.001
Superior frontal gyrus	**−0.114***** (FDR)	**−0.307******	**0.731******	.774	151.829	<.001
Left Heschl's gyrus	**−0.170**** (FDR)	−0.027	**0.610******	.450	36.279	<.001
Occipital fusiform gyrus	**−0.149**** (FDR)	**−0.120***	**0.678******	.573	59.512	<.001
Anterior insula	**−0.133**** (FDR)	**−0.113***	**0.710******	.608	68.805	<.001
Lingual gyrus	**−0.135*** (FDR)	**−0.149****	**0.658******	.553	54.825	<.001
Cerebellum	**−0.132*** (FDR)	**−0.211******	**0.616******	.534	50.720	<.001
Frontal operculum	**−0.130*** (FDR)	**−0.175*****	**0.631******	.529	49.729	<.001
Postcentral gyrus	**−0.114**†	**−0.235******	**0.644******	.580	61.210	<.001
Superior temporal gyrus	−0.061	**−0.262******	**0.745******	.727	117.766	<.001
Posterior cingulate cortex	−0.038	**−0.220******	**0.732******	.662	86.904	<.001
Cerebrospinal fluid						
Lateral ventricles	**0.196***** (FDR)	−0.009	**0.543******	.293	18.379	<.001
Third ventricle	**0.152**+	0.044	**0.448******	.194	10.680	<.001
Fourth ventricle	0.129	−0.053	**0.460******	.215	12.114	<.001

Note: Along with neutrophil count, age and total brain volume were included as additional predictors to control for their effects. See figure 2 and supplementary table 1 for further information. (FDR) = significant after correcting for multiple comparisons with the Benjamini–Hochberg procedure. Statistically significant associations are marked in bold.
+$P = .062$; †$P = .051$; *$P < .05$; **$P < .025$; ***$P \leq .01$; ****$P < .001$.

Overall, considering the results from our study and from previous literature together,[38,42,43] a common mechanism in the etiology of schizophrenia, MS, and AD may be implied, in which neutrophils act as triggers, or one of the most important triggers, of tissue loss from the initial stages of the disease, leading to subsequent cognitive and clinical decline. Recent reports of a common spatial pattern of brain abnormalities between AD and adolescent-onset schizophrenia patients,[57] as well as the finding that patients with schizophrenia are at a higher risk of developing dementia,[58] give support to the hypothesis of shared causative mechanisms between AD and schizophrenia. Moreover, several etiological similarities between MS and schizophrenia have been found,[59] as well as an increased probability of MS patients developing schizophrenia.[59]

Importantly, we carefully examined the impact of potential confounders that may alter neutrophils, such as antipsychotic medication,[60] other psychotropic medications such as lithium,[61,62] BMI, and tobacco or cannabis smoking, since a delay in spontaneous neutrophil death as a consequence of cigarette smoke and nicotine has been observed.[63] None of these factors affected the reported associations between neutrophil count and brain volume or clinical symptomatology, even though lithium intake was associated with increased GM volume in patients, as has been previously reported in healthy people[64] and bipolar patients.[65] Another important factor that we considered is acute infection, since it could increase blood cell count at a particular moment. However, since neutrophil count remained stable and increased in FEP with respect to HC 2 years after the initial blood assessment, it is highly unlikely that an acute infection could explain any of the results presented.

In light of all this, neutrophil count could be postulated as an indicator or potential biomarker of FEP and schizophrenia severity. This indicator would have the advantage of being inexpensive and handy, as it can be quantified with a single blood sampling. Altogether, these results open the door to some therapeutic options concerning neutrophils that have already been proposed for other conditions, such as neutrophil depletion or blocking neutrophil entry into the brain.[18,49] Further research should assess whether therapies of this kind could potentially improve the course of the psychotic disorder or even stop its progression. More research is needed to replicate our results, including the specific brain areas showing reduced volume associated with neutrophils, and which particular consequences it may have. Moreover, future studies may also address many unresolved questions arising from the present results; eg, whether there are specific subtypes of the disease, or age of onset subgroups, in which the association between neutrophils and brain volume and/or clinical symptomatology is particularly relevant; whether there are other blood cell types directly or indirectly involved in this framework, eg, by amplifying

Table 4. Linear Regression Analyses Between Neutrophil Count and Symptomatology, Estimated With the PANSS Scores

Score	Neutrophil Count	Cannabis Smoking	Years of Education	Antipsych. Medication	BMI	R^2	*F* test	*P* Value
Total PANSS	**0.173***	**0.245*****	**−0.187****	—	—	.136	6.966	<.001
Positive PANSS subscale	**0.166***	—	**−0.218*****	**−0.250*****	**−0.200****	.160	6.281	<.001
P1. Delusions	ns	—	**−0.198****	**−0.316******	**−0.169***	.167	6.608	<.001
P2. Conceptual disorganization	**0.163**†**	—	**−0.174***	**−0.212****	**−0.207****	.132	5.008	<.001
P3. Hallucinatory behavior	**0.182***	—	**−0.292******	ns	**−0.238*****	.168	6.642	<.001
P4. Excitement	ns	—	ns	**−0.213****	ns	.068	2.405	.053
P5. Grandiosity	ns	—	ns	ns	ns	—	—	—
P6. Suspiciousness/persecution	**0.216*****	—	**−0.240*****	**−0.223*****	ns	.143	5.491	<.001
P7. Hostility	**0.247*****	—	ns	ns	ns	.062	2.191	.073
Negative PANSS subscale	—	—	**−0.264*****	—	—	.070	10.142	.002
General PANSS subscale	**0.152**+**	**0.296******	—	—	—	.111	8.404	<.001
G1. Somatic concern	ns	ns	—	—	—	—	—	—
G2. Anxiety	ns	**0.182***	—	—	—	.043	2.978	.054
G3. Guilt feelings	ns	ns	—	—	—	—	—	—
G4. Tension	ns	**0.259*****	—	—	—	.082	5.999	.003
G5. Mannerisms and posturing	**0.160**†**	**0.169***	—	—	—	.055	3.872	.023
G6. Depression	ns	ns	—	—	—	—	—	—
G7. Motor retardation	ns	**0.218*****	—	—	—	.066	4.751	.010
G8. Uncooperativeness	ns	**0.234*****	—	—	—	.058	4.094	.019
G9. Unusual thought content	ns	**0.220*****	—	—	—	.068	4.914	.009
G10. Disorientation	ns	**0.345******	—	—	—	.126	9.694	<.001
G11. Poor attention	ns	**0.209****	—	—	—	.056	3.980	.021
G12. Lack of judgment and insight	ns	ns	—	—	—	—	—	—
G13. Disturbance of volition	**0.197****	**0.244*****	—	—	—	.099	7.382	<.001
G14. Poor impulse control	ns	**0.288******	—	—	—	.090	6.599	.002
G15. Preoccupation	**0.283******	**0.188****	—	—	—	.117	8.848	<.001
G16. Active social avoidance	ns	**0.172***	—	—	—	.035	2.452	.090

Note: The analysis of the individual items of the positive PANSS subscale included neutrophil count, years of education, antipsychotic medication, and BMI as predictors. The analysis of the individual items of the general PANSS subscale included neutrophil count and cannabis smoking as predictors. Uncorrected *P* values are presented. Statistically significant associations are marked in bold. ns, nonsignificant.
+$P = .064$; †$P < .06$; *$P < .05$; **$P < .025$; ***$P \leq .01$; ****$P < .001$.

neutrophil response; and, finally, the impact of substance use. Although here we examined the effects of tobacco and cannabis smoking, patients with schizophrenia and FEP tend to be heavy smokers,[66] and even if we assume that smoking does not have a direct impact on amplifying the neutrophil immune response, increasing the amount of circulating neutrophils, in a context in which something is not working properly, would only aggravate the condition.

This study presents some noteworthy strengths. First, the sample employed was relatively large and homogeneous in regard to illness duration, therefore providing more reliability and generalizability to our findings. Second, antipsychotic medication information was thoroughly collected and its potential effects on brain volume or neutrophils were controlled; other potentially confounding factors, such as other psychotropic medications, sex, BMI, and tobacco and cannabis smoking, were controlled as well. Third, we assessed brain changes employing a whole-brain approach, which allowed us to analyze GM and CSF volume changes in an unbiased manner. Conversely, some limitations should also be noted. First, this was an observational study, meaning that we were not able to manipulate the independent variable, and cause–effect relationships cannot be inferred. Second, MRI acquisition and blood sampling were not necessarily carried out the same day; moreover, MRI acquisitions were conducted on 5 different scanners and this might have biased the results. Third, while the effects of antipsychotic medication were controlled for, we did not have information on the precise dosage of other psychotropic medications. Fourth, information on anti-inflammatory medication use, inflammatory diseases, and sleep-related problems, which could have altered neutrophil count, were not recorded. Finally, this was an exploratory study, and we lacked information on more precise inflammatory markers.

In conclusion, we show, for the first time, an association between neutrophil count and reduced GM tissue in FEP. Neutrophil count was also associated with increased CSF volume and higher scores in the total PANSS and several relevant items, such as those assessing hallucinations and avolition. These results suggest that the immunological system of patients with schizophrenia and related

disorders is dysregulated, and appear to give support to the autoimmune hypothesis. Neutrophil count is proposed as an indicator of psychosis severity, and may give rise to new therapeutic options addressing neutrophils or the neutrophil immune response.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin* online.

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